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Award Number: DAMD17-02-2-0061

TITLE: Development and Implementation of an Objective, Non-Invasive, Behaviorally Relevant Metric for Laser Eye Injuries

PRINCIPAL INVESTIGATOR: LTC Cheryl DiCarlo, D.V.M., Ph.D.
Gary Martinsen, O.D., Ph.D.
John D'Andrea, Ph.D.
Harry Zwick, Ph.D.
Bruce E. Stuck

CONTRACTING ORGANIZATION: T.R.U.E. Research Foundation
San Antonio, Texas 78217

REPORT DATE: September 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2004	Annual (1 Sep 2003 - 31 Aug 2004)

4. TITLE AND SUBTITLE
Development and Implementation of an Objective, Non-Invasive, Behaviorally Relevant Metric for Laser Eye Injuries

5. FUNDING NUMBERS
DAMD17-02-2-0061

6. AUTHOR(S)
LTC Cheryl DiCarlo, D.V.M., Ph.D.; Gary Martinsen, O.D., Ph.D.; John D'Andrea, Ph.D.; Harry Zwick, Ph.D.; Bruce E. Stuck

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
T.R.U.E. Research Foundation
San Antonio, Texas 78217

E-Mail: Cheryl.DiCarlo@brooks.af.mil

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT	12b. DISTRIBUTION CODE
Approved for Public Release; Distribution Unlimited	

13. ABSTRACT (Maximum 200 Words)

Background: Battlefield and accidental laser-induced retinal injuries can produce significant retinal scarring and permanent vision loss. Lasers have become an integral part of military weapon system. The proliferation of lasers in battlefield and workplace environments increases the probability that ground and air crews will acquire laser-induced deficits in visual function

Objective: The objective of this research are three-fold. The first, is to use a modification of an existing behavioral technique to assess visual function by establishing acuity and contrast sensitivity thresholds in non-human primates prior to and after laser induced retinal injury. The second is to correlate the results with the functional assessment obtained by multifocal electroretinography (mfERG). This is to determine if mfERG is sensitive enough to provide a sensitive, objective functional metric for laser retinal injury. The third objective is to determine whether the mfERG can assess the recovery from retinal laser injury as measured by the behavioral technique.

Materials and Methods: Four Rhesus monkeys are currently being trained to test their visual acuity and contrast sensitivity using a program that was generated on Labview software. MfERG recordings are being collected on the animals for comparison to the contrast sensivity/visual acuity test results. Retinal laser lesions will be made once all baseline data is collected.

Results and Conclusions: Animals are in the final stages of the behavioral testing program with production of contrast sensitivity curves near completion. MfERG recordings using the 509 hexagonal pattern appear to provide the best sensitivity for retinal laser lesions.

14. SUBJECT TERMS	15. NUMBER OF PAGES
laser, lesio, retina, spatial frequency, contrast sensitivity, multifocal electroretinogram, behavior, non-human primate	25
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

Permanent vision loss can result from battlefield and accidental laser retinal injury. Numerous cases of accidental laser eye injuries have been reported [1], [2], [3], [4], [5], [6], [7]. Despite the increasing risk to military personnel from battlefield and accidental laser exposures, there is still no universally accepted evaluation and treatment regime for laser eye injuries. Additionally there is currently no functional metric to assess recovery from laser eye injury over time that is correlated to retinal morphological metrics currently in use. The objective of this project is to identify the relationship between morphological indexes [e.g., ocular coherence tomography (OCT), confocal Scanning Laser Ophthalmoscopy (cSLO)] of retinal damage and MERG patterns with a known behavioral endpoint (acuity/contrast sensitivity).

Changes have been reported in the electroretinogram waveforms following laser insult in the rabbit [8] and in the nonhuman primate [9], [10], [11]. In the nonhuman primate reports from the U.S. Army Medical Research Detachment (USAMRD), an effect on visual function was seen with small spot laser exposures at levels well below the threshold damage levels [9]. The difficulty in assessing these small spot laser lesion exposures using the conventional ERG technique is related to its measurement of a mass retinal response due to the scatter of light from a traditional flash stimulus. In order to determine the function of several areas of the retina simultaneously, the multifocal electroretinogram MERG was developed by Sutter in 1992. This technique utilizes the M-sequence method to map focal ERGs obtained from multiple areas of the retina. The ERGs are recorded simultaneously and presented as a topographical map [12] [13]. Using devices that are designed for a continuous visualization of the fundus during recording enables the diagnostician to determine local retinal dysfunction from hereditary disease [14], [15], [16], drug induced visual field constriction [17], age-related macular degeneration [18], [19] and inner retinal defects from glaucoma [20]. The Visual-Evoked Response Imaging System (VERIS) Clinic™, a MERG system with continuous visualization of the fundus, can obtain 61 – 241 focal ERG responses per eye within a 4 – 16 minute recording time [21]. Changes in the MERG waveforms with focal injury or disease have been reported [22], [23], [24].Therefore, a potential exists for use of this system in the assessment of function of local areas of the retina exposed to laser insult and quantitative functional assessment of asymmetrical morphological lesion recovery.

Behavioral techniques in conjunction with using a confocal Scanning Laser Ophthalmoscope (cSLO) are used by the Naval Health Research Center (NHRC) Detachment, Brooks City Base as an alternative to current histological techniques for the assessment of visual function. This approach provides a real-time and longitudinal analysis of damage, analysis of repair processes and functional change following laser exposure and damage to the retina. Using an operant conditioning approach with positive (appetitive) reinforcement, subjects learn to respond to position of gap orientation or opening using the Landolt ring as a target stimulus. The Landolt ring was developed for measuring acuity with its unique technical qualities (gap and stroke width = 1/5 diameter of ring) and precise qualities for measuring the visual angle. The response with NHP is generally within 1 second with majority of responses near 300 ms. The task increases in difficulty across trials as the contrast level is lowered and target stimuli are reduced in size. The use of four contrast levels and five different stimulus' sizes provides the necessary data to establish a subject's visual acuity and contrast sensitivity threshold pre- and post-laser exposure. Additionally, by presenting the stimuli in the raster scan of the cSLO allows for concurrent collection of retinal images in an awake nonhuman primate. This approach has been successfully used by the NHRC Detachment in the past [25], [26] to determine subjects' preferred retinal location and to precisely locate laser exposures, which are then evaluated by comparing subjects' behavioral performance data (reaction time and percent correct responses) pre- and post-lesion. Results show that sub-MPE (maximum permissible exposure) laser exposures cause only minimal and transient (flashblindedness and startling) effects on performance, and that parafoveal lesions produce no significant disruption in visual function.

With the numerous risks to vision that military personnel are exposed to on the modern battlefield, it is imperative that new techniques for the long-term functional assessment of the retina for laser eye injury are developed. This protocol is expanding on-going research to include the functional assessment of laser retinal injury through the use of the MERG with the intent of correlating the function to currently used behavioral metrics of the visual system. In addition, this research will expand into the comparison of the functional metrics to the use of morphological techniques, such as fluorescein angiography, scanning laser ophthalmoscopy, and optical coherence tomography.

BODY

Statement of Work Experiment 1

Determine the sensitivity of the multifocal electroretinogram (mfERG) to militarily relevant laser damage.

Research Accomplishment Experiment 1

Normal 103-hexagon values for Rhesus and Cynomolgus monkeys were determined and reported previously in order to compare recordings from laser-lesioned animals for functional recovery [27]. The standard 103-hexagon stimulus array pattern of mfERG recorded approximately a 2-degree area for each hexagon (an unscaled array). Since this averaged recording area was larger than the laser lesions, mfERG recordings using the more sensitive 509-hexagon stimulus array pattern were accomplished using the same recording times as the 103-hexagon pattern. The technique was accomplished and reported [28] (see Appendix) using animals in this concurrent treatment study. The animals were exposed and recorded up to four days post-injury. Two animals were kept until 4 months post-exposure. Only one animal could be recorded out to 4 months since the mfERG unit was non-operational for approximately six (6) months of this grant period. What was seen on animals that were recorded was an increased amplitude of the foveal region when laser lesions were placed in the macula surrounding the fovea. This amplitude increase was seen on the recordings post-injury through Day 4 post-injury. Eventually it diminishes to a more "normal" response by Day 14 post-injury. This increased amplitude response is related to an initial lack of inhibition of the surrounding retinal cells. Please see publication in Appendix for details of this work.

Statement of Work Experiment 2

Identify the relationship between the morphological indices of retinal damage and mfERG patterns with a known behavioral endpoint (visual acuity/contrast sensitivity).

Research Accomplishment Experiment 2

Six pair-housed nonhuman primates have been behaviorally shaped to respond to a change in the direction of the opening of a Landolt C using both eyes (see Appendix Figure 1 for poster presentation describing this set-up). A correct response in contrast led to the presentation of the next level of diminished contrast. An incorrect response increased the contrast level by one unit. Sixteen contrast sensitivity levels are available for presentation to the animal. Spatial frequency was tested using 5 sizes of the Landolt "C." As with contrast, a correct response led to the presentation of the next level of diminished spatial frequency. An incorrect response increased the level (to a larger "C") by one unit. An example of a contrast sensitivity curve for one subject using both eyes is seen in the Appendix (Figure 2).

Statement of Work Experiment 3

Extend the sensitivity of the mfERG metric to extramacular pathologies by extracting a more precise ED_{50} dose (effective dose at 50%) for mfERG and OCT changes.

Research Accomplishment Experiment 3

Concurrent laser-injury treatment studies are underway and future studies are being developed utilizing cynomolgus monkeys. Argon (514 nm) and Nd:YAG (1064 nm) retinal laser lesions will be produced to determine a more precise ED50 using the mfERG and OCT findings compared to histopathology. The laboratory is also in the process of procuring an updated OCT/SLO system with improved resolution to aid in this work for comparison to morphological techniques. Utilizing the Cynomolgus monkeys from other projects is a more reasonable approach then utilizing the highly valuable behaviorally trained Indian-origin Rhesus monkeys on this study for histopathology.

KEY RESEARCH ACCOMPLISHMENTS

1. mfERG recordings collected using the 509-hexagon pattern for increased sensitivity in detecting laser-lesions. A modification to this collection procedure with increased recording time is underway. The mfERG was inoperable for 6 months out of this grant period. It had to be sent back to the sole developer for repairs.

2. Six Rhesus monkeys are currently chair-trained and operant conditioning is completed for the visual acuity/contrast sensitivity program. Contrast sensitivity curves for each animal (testing both eyes) are complete. Testing of individual eyes is underway and must be completed before laser-retinal injury can be produced.

REPORTABLE OUTCOMES

Publications

C.D. DiCarlo, J. Brown, A. Grado, J. M. Sankovich, H. Zwick, D. Lund, B. E. Stuck, "The Use of the Multifocal Electroretinogram (mfERG) for assessing the response of 670 nm Light Emitting Diode (LED) Photoillumination in an Animal Model with Laser Retinal Injuries," Proceedings of Lasers and Biophotonics in Veterinary Medicine, Lasers in Surgery: Advanced Characterization, Therapeutics, and Systems XIV, International Society for Optical Engineering, Society of Photo-Optical Instrumentation Engineers (SPIE), January 2004, Volume 5 (1), ISSN 1605-7422, SPIE paper #5312-67, p.341-363

J.Brown, C.D. DiCarlo, H. Zwick, D.J. Lund, J.M. Sankovich, S.T. Schuschereba, B.E. Stuck, "Multifocal ERG responses following acute retinal laser photocoagulation show increased amplitudes and delayed implicit times," Association for Research in Vision and Ophthalmology (ARVO), April 2004, Abstract #2029.

Presentations

C.D. DiCarlo, "Medical Countermeasures for Laser Eye Injury: Multifocal Electroretinography, A New Functional Metric for Assessing Therapies of Retinal Injury," Presentation to the U.S. Army Medical Research and Materiel Command, Medical Technology IV Review Panel, Lansdowne, VA, 21 October 2003

C.D. DiCarlo, J. Brown, A. Grado, J. M. Sankovich, H. Zwick, D. Lund, B. E. Stuck, "The Use of the Multifocal Electroretinogram (mfERG) for assessing the response of 670 nm Light Emitting Diode (LED) Photoillumination in an Animal Model with Laser Retinal Injuries," Proceedings of Lasers and Biophotonics in Veterinary Medicine, International Society for Optical Engineering, Society of Photo-Optical Instrumentation Engineers (SPIE), 26 January 2004

C.D. DiCarlo, "Use of the mfERG for assessing the response of 670 nm LED photoillumination in an animal model with laser retinal injuries," 25th Annual Lasers on the Modern Battlefield Conference, Brooks City-base, San Antonio, Texas, 25 February 2004.

J.Brown, C.D. DiCarlo, H. Zwick, D.J. Lund, J.M. Sankovich, S.T. Schuschereba, B.E. Stuck, "Multifocal ERG responses following acute retinal laser photocoagulation show increased amplitudes and delayed implicit times," Association for Research in Vision and Ophthalmology (ARVO), Fort Lauderdale, Florida, 26 April 2004, poster #B840.

C.D. DiCarlo, A. Grado, J. Sankovich, T. Garza, J. Morin, H. Zwick, B. E. Stuck, "The Use of the Multifocal Electroretinogram for Assessing Functional Damage to the Retina from Laser Injury, "The Peer-Reviewed Medical Research Program Conference, San Juan, Puerto Rico, 26 – 28 April 2004, poster board #M4.

Training and Travel

Primate Training and Enrichment Workshop, University of Texas, MD Anderson Cancer Center, Bastrop, Texas, 23 September 2003 – 27 September 2003 (Dr. DiCarlo and SPC Grado)

Texas Branch Chapter of the American Association of Laboratory Animal Science (TBAALAS) meeting, San Antonio, Texas, 28 – 30 April 2004 (behavioral shaping technicians - SPC Grado, Mr. Garza, Mr. Morin, Ms. Henry and Mr. Kosub)

Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, Fort Lauderdale, Florida, 24 – 29 April 2004 (Dr. J. Brown)

CONCLUSIONS

Permanent eye damage can produce decrements in visual function that change over the time course of the healing process. Our understanding of laser eye injury damage, treatment and recovery mechanisms will be enhanced by our ability to observe both subtle and well-defined changes in retinal morphology and their relation to visual function over time. Additionally, a functional metric or endpoints for the development of a treatment for laser-induced retinal injuries is needed. The use a behavioral technique for assessment of visual function in alert non-human primates (NHP) to measure the loss of visual function due to laser exposure and to compare this assessment with that of multi-focal electroretinography (mfERG) is on-going. The mfERG is a functional measure of the visual system at the retinal level, whereas contrast sensitivity and visual acuity assess the entire visual system and its ability to compensate. Behavioral measures of visual function are currently underway to determine whether mfERG provides an objective, non-invasive metric of retinal damage to quantitatively assess the natural time course of recovery from laser-induced retinal injury. mfERG shows promise for an assessment of retinal areas with large lesion patterns (300 – 500 microns) but utilizing the standard 103 hexagon pattern the mfERG is not sensitive enough to accurately localize discreet small laser lesions. Investigation of the appropriate collection time for the 509-hexagon pattern is on-going to optimize the recordings for this and our other concurrent research studies.

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APPENDIX

Use of the multifocal electroretinogram (mfERG) for assessing the response of 670 nm light emitting diodes (LED) photoillumination in an animal model with laser retinal injuries.

Cheryl D. DiCarlo[*a], Jeremiah Brown[b], Andres Grado[a], James Sankovich[a], Harry Zwick[a], David J. Lund[a], and Bruce E. Stuck[a]
[a]U.S. Army Medical Research Detachment, Walter Reed Army Institute of Research, 7965 Dave Erwin Drive, Brooks City-base, TX 78235-5108
[b]Northrop Grumman Information Technology, 4241 Woodcock Drive, Suite B100, San Antonio, Texas 78228

ABSTRACT

There is no uniformly accepted objective method to diagnose the functional extent of retinal damage following laser eye injury and there is no uniform therapy for laser retinal injury. J.T. Eells, et al, [1] reported the use of Light Emitting Diodes (LED) photoillumination (670 nm) for methanol-induced retinal toxicity in rats. The findings indicated a preservation of retinal architecture, as determined by histopathology and a partial functional recovery of photoreceptors, as determined by electroretinogram (ERG), in the LED exposed methanol-intoxicated rats. The purpose of this study is to use multifocal electroretinography (mfERG) to evaluate recovery of retinal function following treatment with LED photoillumination in a cynomolgus monkey laser retinal injury model. Control and LED array (670 nm) illuminated animals received macular Argon laser lesions (514 nm, 130 mW, 100 ms). LED array exposure was accomplished for 4 days for a total dose of 4 J/cm^2 per day. Baseline and post-laser exposure mfERGs were performed. mfERG results for five animals post-laser injury but prior to treatment (Day 0) showed increased implicit times and P1 waveform amplitudes when compared to a combined laboratory normal and each animal's baseline normal values. In general, preliminary mfERG results of our first five subjects recorded using both the 103-hexagon and 509-hexagon patterns indicate a more rapid functional recovery in the LED illuminated animal as compared to the control by the end of the fourth day post-exposure. Research is continuing to determine if this difference in functional return is seen in additional subjects and if statistical significance exists.

Keywords: multifocal electroretinogram, mfERG, laser retinal injury, nonhuman primate

1. INTRODUCTION

Although controversy exists over the use of low intensity monochromatic light therapy for wound healing [2, 3], there is a tremendous interest in the use of light emitting diode (LED) arrays for medical therapy. The reasons for controversy may include no standardized protocols for treatment along with only theorized mechanism of action of the photoillumination. The mitochondrial cytochrome oxidase system is discussed in the literature as a possible photoacceptor of light in the red to near infrared range[4-6]. Recent in-vitro and in-vivo studies from the Medical College of Wisconsin indicate promising effects of the use of 670 nm LED arrays for nervous and retinal tissue rescue from toxins[1, 7]. Neuronal ATP content of cultured neurons exposed to potassium cyanide was rescued by LED using 4 joules/cm^2 in the in-vitro study. In the in-vivo work using the same exposure parameters of 4 joules/cm^2, a partial functional recovery of photoreceptors, as determined by electroretinogram (ERG), was seen in LED exposed methanol-intoxicated rats. These recent successes in the use of LED arrays at 670 stimulated the interest of this laboratory to investigate this modality as a possible therapy for laser eye injury. In order to test a therapeutic agent's effect, the anatomical and functional response of the retina must be addressed.

*cheryl.dicarlo@brooks.af.mil; phone 210-536-4623;fax 210-536-3450

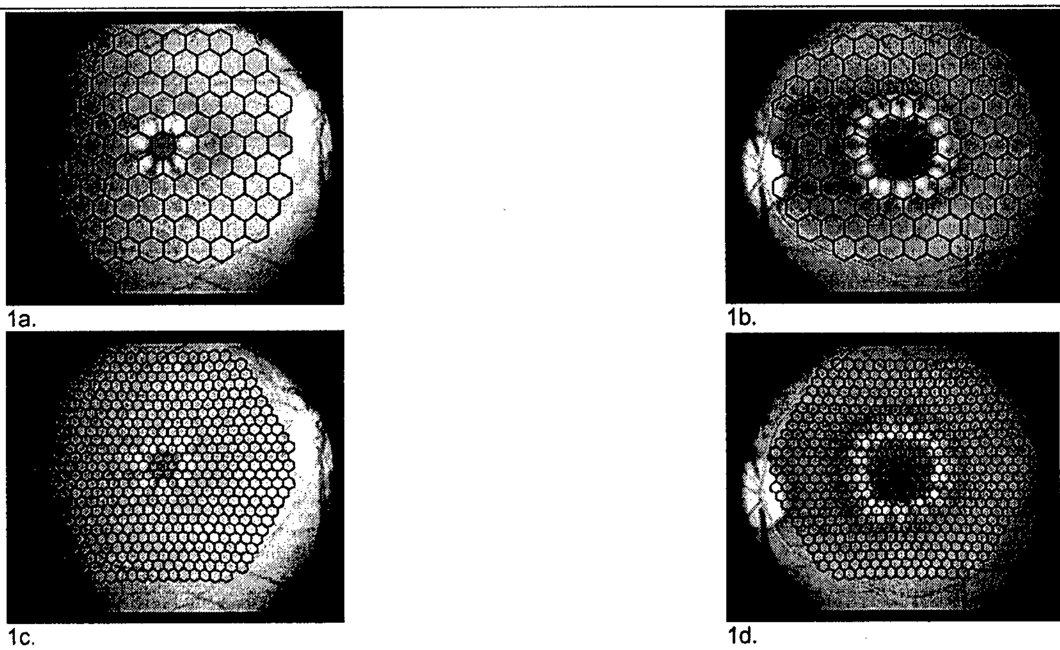
Visual function tests such as contrast sensitivity and visual acuity exams are in use at U.S. Army Medical Research Detachment (USAMRD) for post-laser injury patients. The literature cites attempts to use electroretinography (ERG) following laser insult in the rabbit [8] and in the nonhuman primate [9-11]. In the nonhuman primate reports from the USAMRD, an effect on visual function was seen with small spot laser exposures at levels well below the threshold damage levels [10]. The difficulty in assessing these small spot laser lesion exposures using the conventional ERG technique is related to its measurement of a mass retinal response due to the scatter of light from a traditional flash stimulus. In order to determine the function of several areas of the retina simultaneously, the multifocal electroretinogram (mfERG) was developed by Sutter in 1992. This technique utilizes the M-sequence method to map focal ERGs obtained from multiple areas of the retina. The ERGs are recorded simultaneously and presented as a topographical map [12]. The mfERG waveform of the first order response, which records mainly outer retinal segments, is divided into three basic peaks called N1 (negative deflection), P1 (positive peak) and N2 (negative deflection). Some homology exists between the conventional ERG and the mfERG waveform but a simple correlation to specific classes of retinal cells still cannot be assumed[13]. The purpose of this work is to use the multifocal electroretinography (mfERG) to evaluate the functional damage to the retina post laser insult with and without photoillumination using an LED array.

2. METHODOLOGY

2.1 Animal Preparation/Laser and LED Array Exposure: Five cynomolgus monkeys (2.5 – 4.5 kg) housed under standard laboratory conditions (12 hours light/12 hours dark) were used in this study. All animals involved in this study were procured, maintained, and used in accordance with the Federal Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council. The Air Force Research Laboratory (AFRL) at Brooks City-base, Texas, has been fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International, (AAALAC) since 1967. USAMRD/WRAIR is AAALAC accredited through AFRL. Using a standard protocol reported elsewhere[14], all animals received local (marcaine/lidocaine peribulbar injections) and general anesthesia (propofol via syringe pump) to stabilize eye movement for the laser exposure. Two control and three LED array illuminated animals received macular Argon laser lesions (514 nm, 130 mW, 100 ms) in a circular pattern 500 microns from the center of the fovea in the right eye (OD) and 1000 microns from the center of the fovea in the left eye (OS). The laser-damaged retinas of the three experimental monkeys were exposed to a LED (670 nm) array daily for 4 days at 4 J/cm^2 per day. Sham controls were positioned in front of the non-illuminated LED array for an equal duration of time.

2.2 Multifocal Electroretinogram Recordings: Baseline and post-laser exposure mfERGs were performed on the animals on Day 0, Day 2 and Day 4. Day 0 recordings were obtained prior to LED array or sham exposure. A stimulus array of 103 hexagons, and in some cases 509 hexagons, were evaluated for their sensitivity to detect the laser lesions (400 to 500 microns in diameter) and to follow the lesion response over the next 4 days post-exposure. One of the three LED exposed animals was followed by mfERG out to 4 months post-exposure. Specially made Burian-Allen electrodes (Hansen Ophthalmic Development Lab, Coralville, IA) were placed on the anesthetized (proparacaine drops) corneas of the anesthetized monkeys for the recording process. mfERGs were collected from the monkeys using the Visual-Evoked Response Imaging System (VERIS) Clinic™, a mfERG system with continuous visualization of the posterior segment of the eye. The VERIS system stimulus pattern is projected through a fundus camera at a distance of approximately 4 cm from the animal's cornea. A 103 or 509 focal ERG response recording per eye was collected using an unscaled hexagonal array at a luminance setting of approximately 1:300 within a 3:38 minute recording time (Figures 1a – d). Using the VERIS system software for artifact removal, normal files were created for 103- and 509-hexagon stimulus patterns for comparison to each animal's baseline and post-exposure mfERG recordings. The mfERG recordings were evaluated based on regions using a ring structure for the 103- and 509-hexagonal stimulus pattern (Figures 2a and b). The amplitudes and implicit times were averaged based on the rings associated with and central to the laser lesioned areas. For the right eye, rings 1 and 2 were averaged for the 103-

hexagon pattern and rings 1 through 3 were averaged for the 509-hexagon pattern. The left eye averages included rings 1 through 3 for the 103-hexagon pattern and rings 1 through 5 for the 509-hexagon pattern. Additional non-invasive diagnostics, such as digital fundus photography for red-free and fluoresce in angiography, optical coherence tomography (OCT) and confocal scanning laser ophthalmoscopy (cSLO) were also performed to view the anatomical changes within the tissue. The results of this imaging will not be addressed in this paper.



Figures 1
Right fundus (a.) and left fundus photo (b.) with circular Argon (514 nm) laser lesions and 103-hexagon stimulus pattern overlay. Right fundus (c.) and left fundus photo (d.) with circular Argon (514 nm) laser lesions and 509-hexagon stimulus pattern overlay. Right eye lesions (a. and c.) at 500 microns from fovea. Left eye lesions (b. and d.) at 1000 microns from fovea.

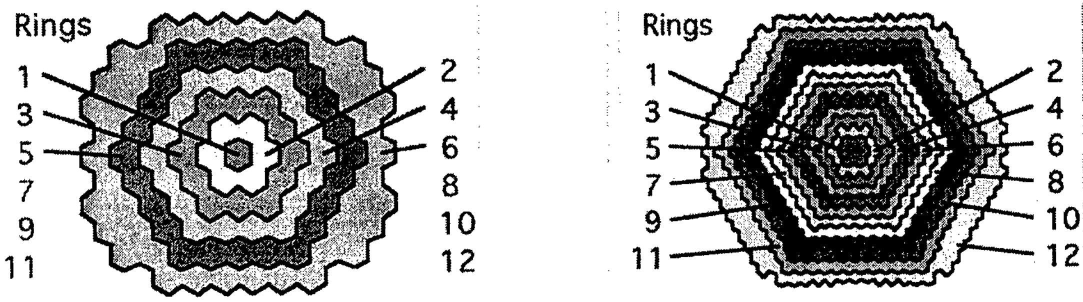


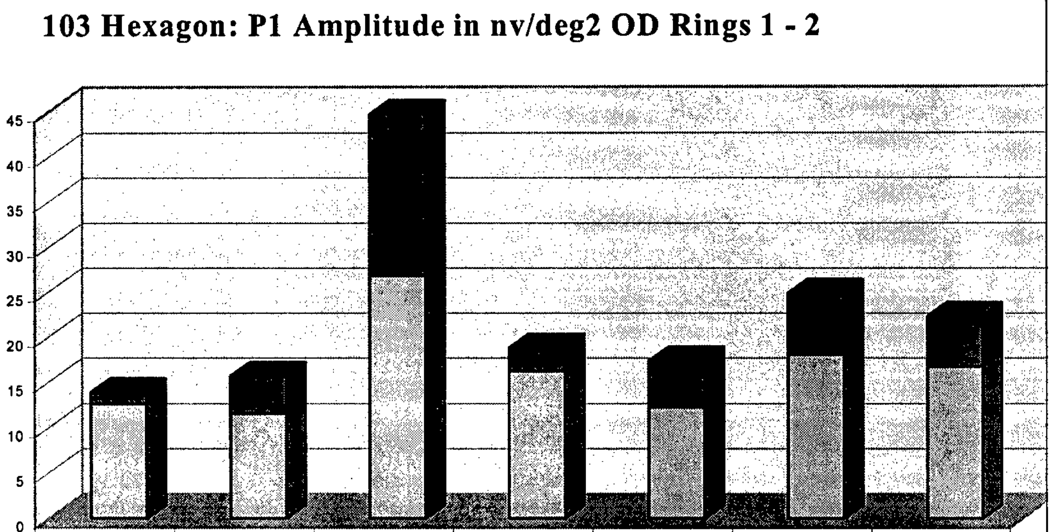
Figure 2
Ring structure of stimulus pattern used for analysis of the 103-hexagon pattern (a.) and the 509-hexagon pattern (b.) These images are from the VERIS™ mfERG software.

3. DATA

The data was graphed using Microsoft Excel™ after averaging the responses for each group from the 1st two rings (right eye – Tables 1a., 3a., 5a., 7a.) and the first three rings (left eye – Tables 1b., 3b., 5b., 7b.)

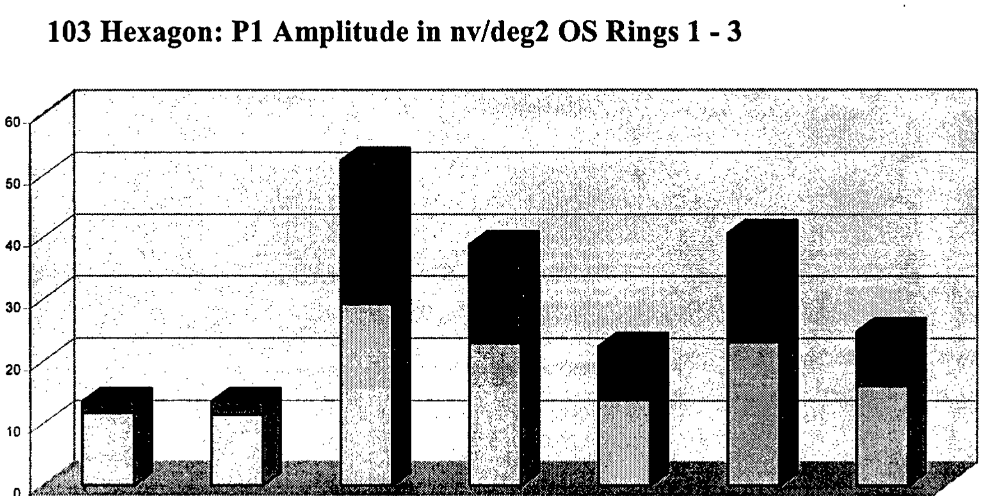
of the 103-hexagonal stimulus array for the P1 amplitude (Table 1) and the implicit times of waveforms N1 (Table 3), P1 (Table 5) and N2 (Table 7). For the 509-hexagonal pattern, the first three rings were averaged (right eye – Tables 2a., 4a., 6a., 8a.) and the first five rings (left eye – Tables 2b., 4b., 6b., 8b.) for the P1 amplitude (Table 2) and the implicit times of waveforms N1 (Table 4), P1 (Table 6) and N2 (Table 8).

Tables 1



	Normal	Baseline	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	1.3	4.2	17.9	2.6	5.2	6.9	5.6
Average	12.6	11.5	26.8	16.2	12.2	18	16.7

a.



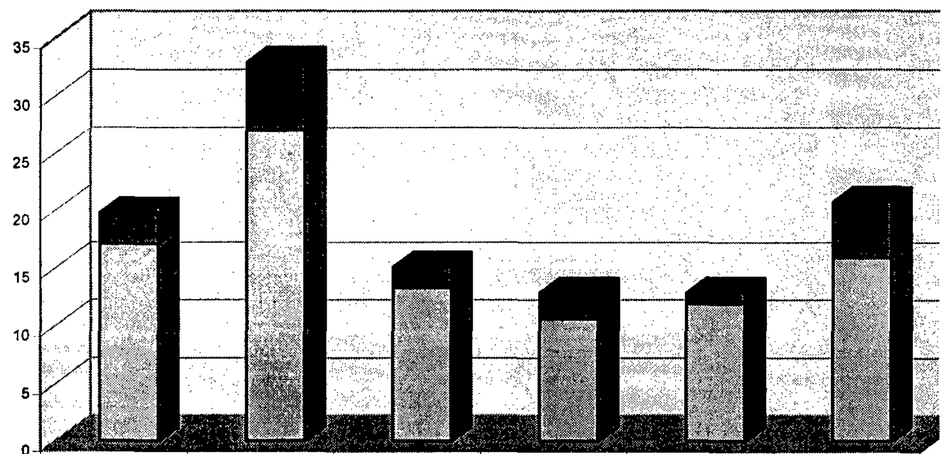
	Normal	Baseline	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	1.9	2.1	23.1	15.9	8.6	17.5	8.7
Average	11.7	11.5	29.2	22.9	13.8	23.1	16.1

b.

Table 2

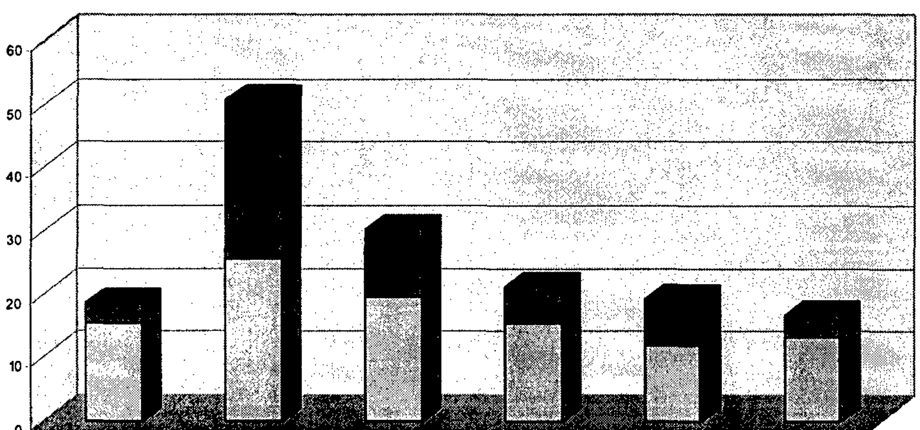
a.

509 Hexagon: P1 Amplitude for Rings 1-3 OD in nv/deg2



	Normal	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	2.7	5.9	1.8	2.3	1	4.9
Average	17.1	27	13.3	10.6	11.9	15.9

509 Hexagon: P1 Amplitude for Rings 1-5 OS in nv/deg2

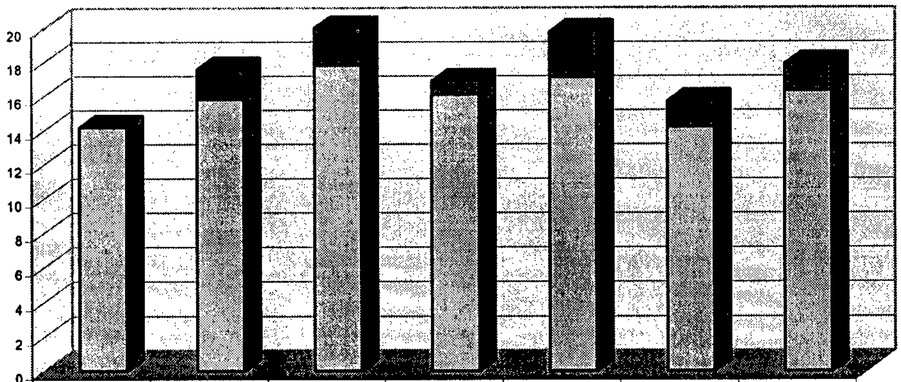


	Normal	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	3.1	25.5	10.9	5.6	7.3	3.7
Average	15.3	25.3	19.3	15.3	11.9	13

b.

Table 3

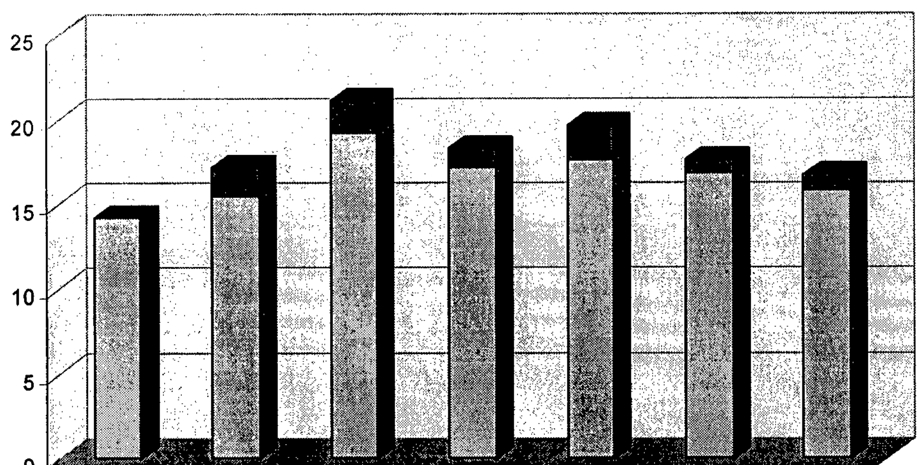
103 Hexagon: N1 Implicit Time in Milliseconds OD Rings 1 - 2



	Normal	Baseline	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	0	1.8	2.2	0.8	2.7	1.5	1.6
Averages	14.2	15.8	17.8	16.1	17.1	14.2	16.3

a.

103 Hexagon: N1 Implicit Time in Milliseconds OS Rings 1 - 3

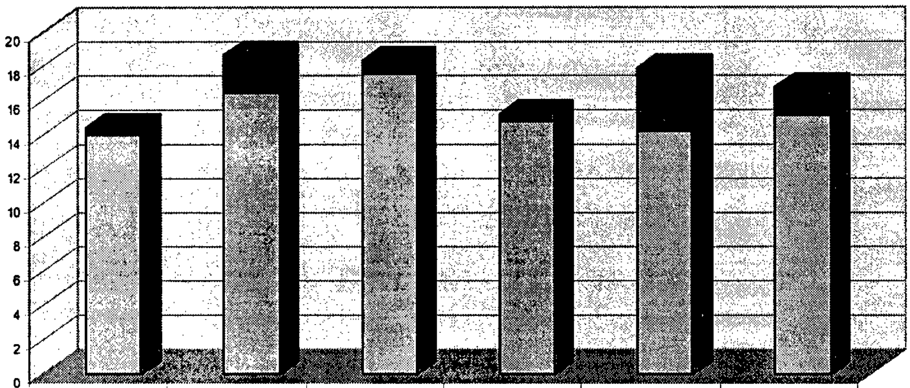


	Normal	Baseline	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	0	1.7	1.9	1.1	2	0.8	0.9
Averages	14.2	15.4	19.2	17.2	17.6	16.8	15.8

b.

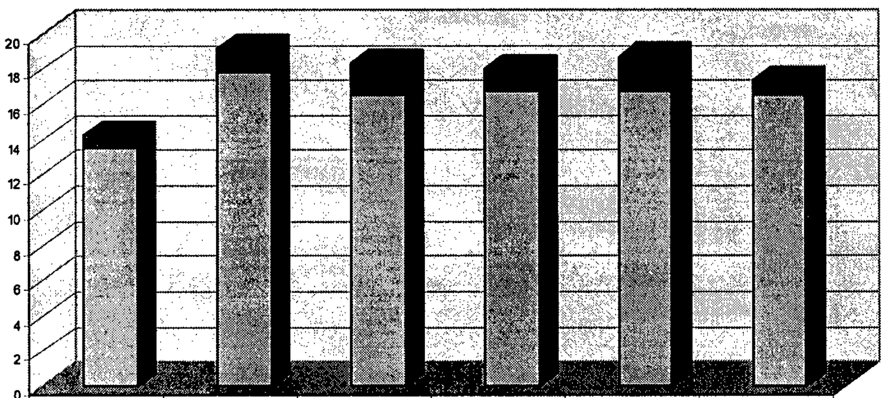
Table 4
a.

509 Hexagon: N1 Implicit Time for Rings 1-3 OD in Milliseconds



	Normal	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	0.5	2.2	0.8	0.5	3.6	1.7
Averages	13.9	16.4	17.5	14.7	14.2	15

509 Hexagon: N1 Implicit Time for Rings 1-5 OS in Milliseconds

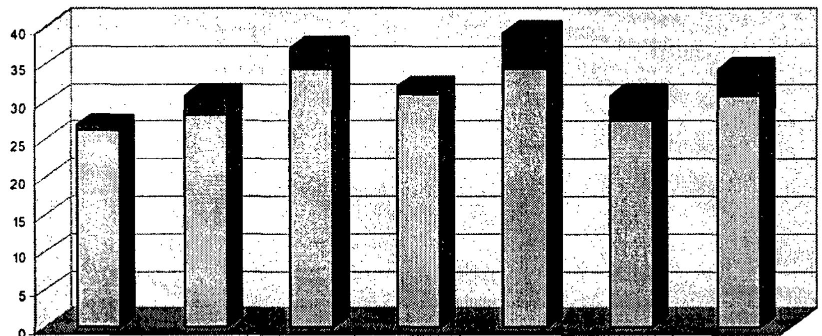


	Normal	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	0.7	1.4	1.8	1.3	2	0.9
Averages	13.5	17.8	16.5	16.7	16.7	16.5

b.

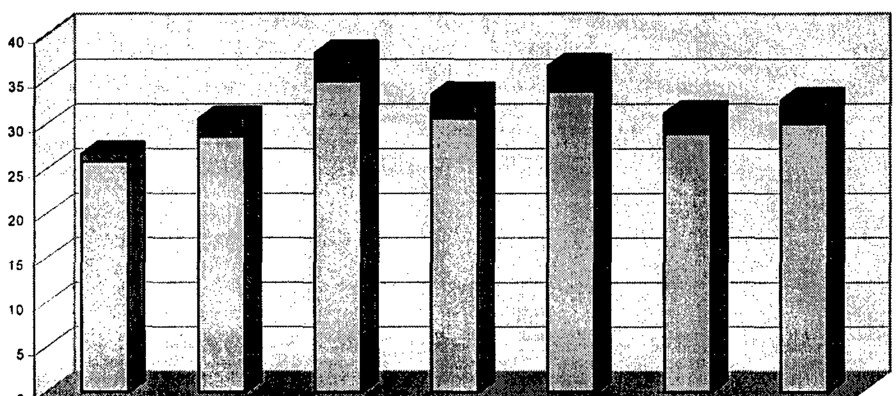
Table 5
a.

103 Hexagon: P1 Implicit Time in Milliseconds OD Rings 1 - 2



	Normal	Baseline	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
■ Standard Deviation	0.6	2.6	2.9	1	4.8	3.4	3.5
▨ Average	26.3	28.3	34.3	31	34.4	27.5	30.9

103 Hexagon: P1 Implicit Time in Milliseconds OS Rings 1 - 3



	Normal	Baseline	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
■ Standard Deviation	0.9	2	3.2	2.7	2.9	2.3	2.7
▨ Average	25.8	28.6	34.8	30.6	33.6	28.9	30

b.

Table 6

509 Hexagon: P1 Implicit Time for Rings 1-3 OD in Milliseconds

	Normal	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	0	2	3.3	0.5	0.5	3.1
Averages	25	32	30.3	30.3	22.8	32.8

a.

509 Hexagon: P1 Implicit Time for Rings 1-5 OS in Milliseconds

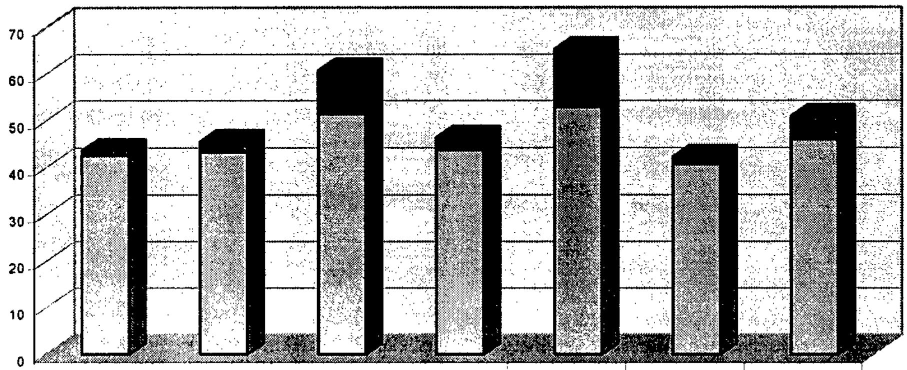
	Normal	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	0	1.1	2.8	0.8	1.9	3.8
Averages	25	31.8	29.3	31.2	28.6	30.5

b.

Table 7

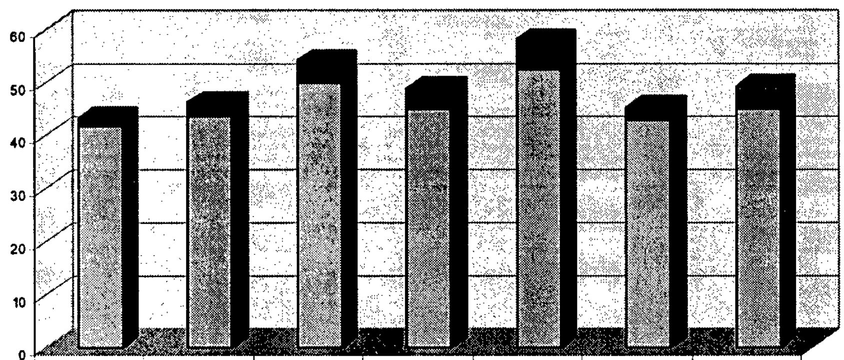
a.

103 Hexagon: N2 Implicit Time in Milliseconds OD Rings 1 - 2



	Normal	Baseline	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	1.1	2.3	9.4	2.7	12.4	1.7	4.9
Average	42.5	43.3	51.4	43.7	52.9	40.6	46

103 Hexagon: N2 Implicit Time in Milliseconds OS Rings 1 - 3



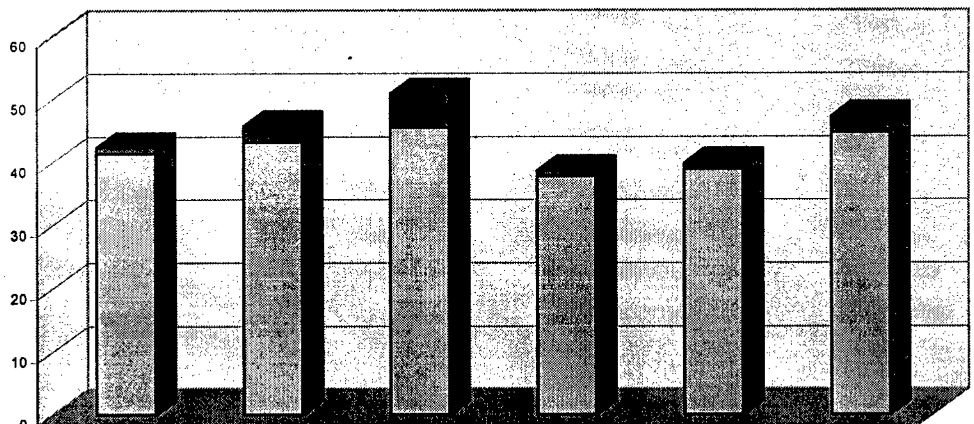
	Normal	Baseline	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	1.6	2.6	4.1	3.9	5.5	2.5	4.3
Average	41.7	43.6	50	45	52.6	42.8	44.9

b.

Table 8

a.

509 Hexagon: N2 Implicit Time for Rings 1-3 OD in Milliseconds



	Normal	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	1	2.6	5.4	0.9	1	2.6
Average	41.4	43.2	45.5	37.8	38.9	44.7

509 Hexagon: N2 Implicit Time for Rings 1-5 OS in Milliseconds

	Normal	Day 0	Day 2 LED	Day 2 Sham	Day 4 LED	Day 4 Sham
Standard Deviation	1	2.2	4	1	3.4	1.4
Average	40.8	47.1	43	44.6	42	46.5

b.

4. RESULTS

From the tables listed above, the normal column refers to the laboratory "normal" for that species within our laboratory and is an "n" of 12 records for the 103-hexagon values and an "n" of 16 for the 509-hexagon values. Whereas, the baseline value refers to the animals included in this study prior to laser lesion exposure. Baseline 509-hexagon recordings were not obtained on the animals included in this study thus far, so these exposed animals were compared to our laboratory baselines for the 509-hexagon "normal." The 103-hexagon recordings are from 5 animals for Day 0; Day 2 columns include recordings from 3 LED exposed animals and 2 sham control animals; Day 4 columns include recordings from 2 LED exposed animals and 2 sham control animals. The 509-hexagon recordings are from 2 animals for Day 0; Day 2 and Day 4 columns include recordings from 1 LED exposed animal and 1 sham control animal. The mfERG 103-and 509-hexagon P1 amplitudes were 2 – 3 times as great immediately post-exposure (Day 0). The 103-hexagon recordings for P1 show slightly higher amplitude averages for the LED exposed animals by Day 4 (18+/-6.9 nV OD and 23.1+/-17.5 nV OS versus 16.7+/-5.6 nV OD and 16.1+/-8.7 nV OS per degree^2 – Table 1) whereas the 509-hexagon recordings yielded lower average values for the LED exposed when compared to the sham control (11.9+/-1 nV OD and 11.9+/-7.3nV versus 15.9+/-4.9 nV OD and 13+/-3.7 nV OS per degree^2 - Table 2). The implicit times on the 103-hexagon and 509-hexagon recordings for all waveforms (N1, P1 and N2) were shorter in the right eye of the LED exposed animals than in the sham controls by Day 4 (Tables 3a. – 8a). The implicit times on the 103-hexagon and 509-hexagon recordings for N1 waveforms were higher in the left eye of the LED exposed animals (16.8+/-0.8 ms, 103 – Table 3b. and 16.7+/-2 ms, 509 – Table 4b.) than in the sham controls (15.8+/-0.9 ms, 103 – Table 3b. and 16.5+/-0.9 ms, 509 – Table 4b.) by Day 4. Whereas, the P1 and N2 implicit time average values were on the 103-hexagon and 509-hexagon recordings were shorter in the left eye of the LED exposed animals than in the sham controls by Day 4 (Tables 5b - 8b.).

5. CONCLUSIONS

Retinal function was assessed using mfERG following treatment with LED photoillumination in a cynomolgus monkey model of laser retinal injury. In our model, a dramatic rise in the amplitude and implicit time of the mfERG waveform was seen immediately post-laser exposure (Day 0 values). These changes in amplitude and implicit time of the right eye recordings were concentrated mainly in the first through the second ring for the 103 hexagon pattern and the first through the third ring of the 509 hexagon pattern. The changes in the left eye recordings were concentrated in the first through the third ring of the mfERG for the 103 hexagon pattern and the first through the fifth ring of the 509 hexagon pattern. Interestingly, the first ring was not an area with a laser insult yet, it produced the most marked increase in amplitude and decrease in implicit time at Day 0. The cause of this effect is unkown but may represent excess neurotransmitter release from the laser damaged areas, a resultant lack of modulatory inhibition of the foveal region, increased reflectivity of the adjacent large white laser lesions or a combination of these possibilities. By Day 2 in most cases, there were apparent differences in the animals receiving the LED array exposures overall with a more rapid return to normal values by Day 4. The N1 average value differences seen in the left eye of the LED exposed as compared to the sham controls were minimal, and probably insignificant. The more rapid return of function overall in the LED exposed animals may indicate a selective action on the mitochondria of specific retinal cells that make up the N1 through N2 waveforms. The current inability to determine statistical significance in the LED exposed groups versus the sham controls is due to the small numbers of subjects studied to date. However, the mfERG did provide a useful functional metric beyond the current anatomical methods in use at our laboratory (funus photography, OCT, cSLO). Since there exists a need for an objective tool to assess retinal function after laser injury and during therapy, the VERIS™ System appears to hold potential to become this unique non-invasive diagnostic functional tool. Follow-up work currently underway at our laboratory will determine the correlation of this methodology to behavioral visual tests in order to fully assess mfERGs functional value for response to laser retinal injury therapy.

ACKNOWLEDGEMENTS

The authors would like to thank SFC Ronald Dicks, SSG Araceli Brown, SPC Osman Hamza and SPC Rachael Cheramie for technical support. Additionally, we thank Mr. Andre Akers for expert photographic support. Grants from the Defense Advanced Research Program Agency (DARPA) and the US Army Medical Research and Materiel Command (DAMD17-02-2-0061) supported this work.

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APPENDIX FIGURES

Figure 1

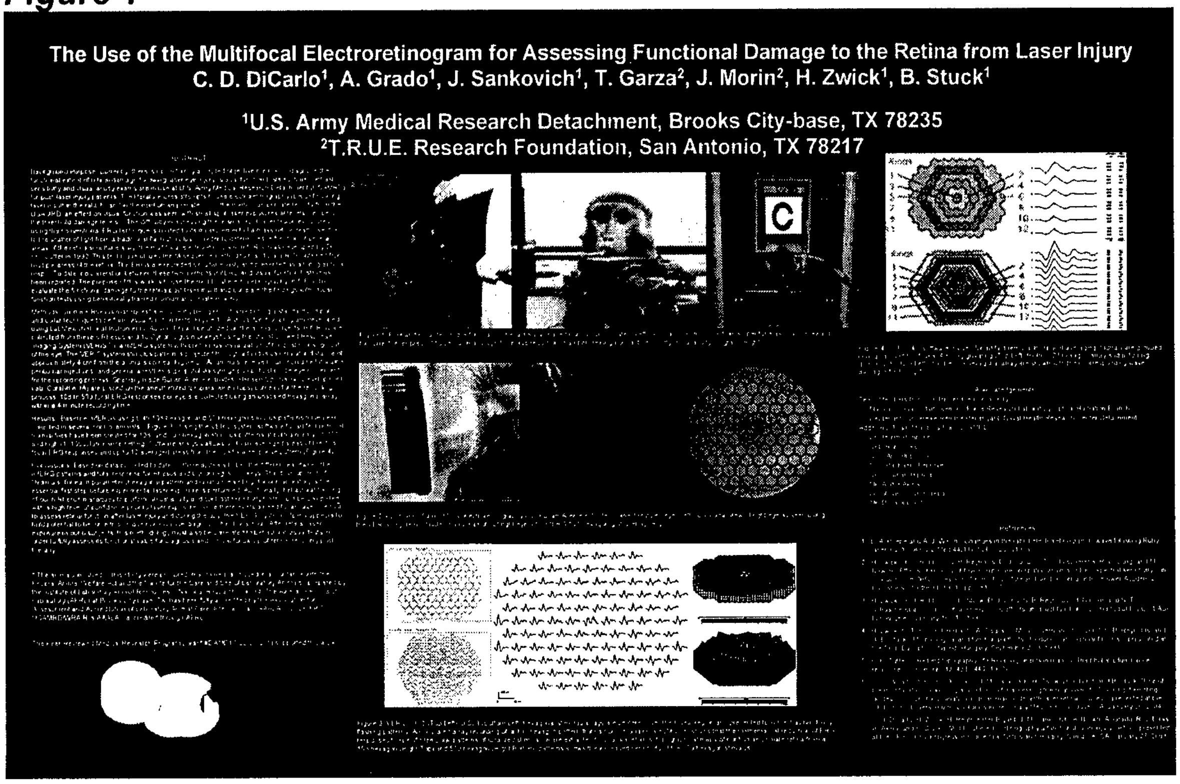

Figure 1 of the poster shows the operant conditioning set-up with positive reinforcement.
Figure 2 of the poster shows the mfERG set-up for collection of recordings.
Figure 3 of the poster shows the 103 and 509-hexagon patterns, 103-hexagon recordings and 103 and 509-hexagon topographical maps of recordings.

Figure 2

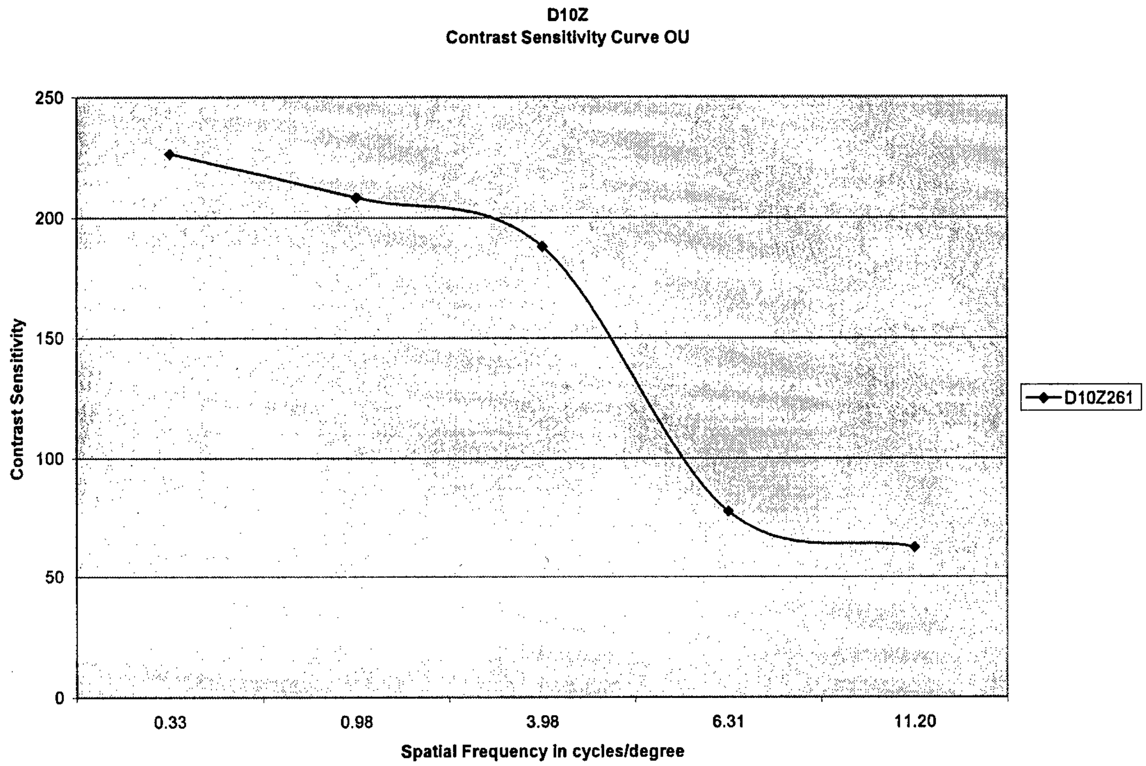

Figure 2 shows a contrast sensitivity/spatial frequency curve using both eyes. The lowest contrast levels (highest number – 226.7) are seen with the largest "C" sizes (lowest numbers – 0.33). As the "C" size diminishes to the smallest "C" (11.20), the contrast (62.5) must be higher in order to visualize.